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SJB file

PROGRESS REPORT

Cooperative Agreement No. 19-209
1/1/76 - 3/31/76

Cold weather and wet forest roads made the field collection of SPB very difficult. This lack of SPB resulted in no progress since last report. Fortunately the incidence of SPB infestation is increasing and bolts are available now. Production procedures are now evaluated and will be ready to report on the 6/31/76 progress report.

Sincerely,

James E. Marler
Division of Biological Sciences
LSU at Alexandria

JEM/mrb

cc: Dr. Stan Barras
Forest Service

Study Plot folder

LOUISIANA STATE UNIVERSITY AT ALEXANDRIA

ALEXANDRIA • LOUISIANA • 71303

March 15, 1976

DIVISION OF SCIENCES
TEL. 445-3672 EXT. 61

Mrs. M. A. Bialas, Chief
Financial Management
United States Department of Agriculture
Forest Service
Room T-10210, U.S. Postal Services Bldg.
701 Loyola Ave.
New Orleans, LA 70113

file

Re: Agreement No. 19-209
Bacteria Free ~~Beetles~~

Dear Mrs. Bialas:

SJB 3/23

Enclosed please find a progress report on the study for the calendar quarter ending December 31, 1975.

I have also submitted a copy to Dr. Stan Barras, U.S. Forestry Service, Pineville.

Sincerely,

James E Marler

Associate Professor of Biology
LSUA

mrb

QUARTERLY PROGRESS REPORT

October 1 - December 31, 1975

Cooperative Agreement No. 19-209

Production of Southern Pine Beetles Free of Microbes in the Gut for Use in Studies on Source of Pheromones

Pupae were gathered from field-collected bolts and surface sterilized. These pupae were allowed to molt to callow adults on water agar plates. The callow adults were placed in an artificial diet containing various antibiotics and allowed to burrow until they emerged. Upon emergence, the beetles were assayed for the presence of microbes. Preliminary results indicate the procedure involved is very effective in producing microbe-free SPB.

TEST DATA

Five bacteria were used to test the effectiveness of streptomycin and garamycin.

The bacteria were chosen to represent a cross section of bacteria encountered in the pine beetle gut and gallery. The bacteria chosen were: Bacillus cereus, a gram-positive rod; Micrococcus luteus, a gram-positive sphere; Serretia marcescens, a gram-negative rod commonly associated with SPB; Enterobacter aerogenes, a gram-negative rod commonly found in the SPB gut; Pseudomonas aeruginosa, a gram-negative rod noted for its resistance to antibiotics. The bacteria were streaked on tryptic soytone agar containing the above antibiotics, in duplicate series.

The test concentrations were 10, 20, 50, 100, and 200 mcg/ml for each antibiotic.

	Streptomycin mcg/ml						Garamycin mcg/ml				
	Control	200	100	50	20	10	200	100	50	20	10
BC	++	-, +	+, +	++, ++	++, ++	++, ++	-, -	-, -	-, +	+, +	+, ++
ML	++	-, +	-, +	++, ++	++, ++	++, ++	-, -	-, -	-, +	+, +	++, ++
SM	++	-, -	+, +	++, ++	++, ++	++, ++	-, -	-, -	-, -	-, -	+, ++
EA	++	-, -	-, -	+, +	-, +	+, ++	-, -	-, -	-, -	-, -	-, +
PA	++	++, ++	++, ++	++, ++	++, ++	++, ++	-, -	-, -	+, -	+, +	++, ++

++ = good growth

+ = fair growth

- = no growth

The results indicate garamycin at 100 mcg/ml to be the best antibiotic.

Sorbic acid was tested against 6 fungi and yeast. These were Ceratocystis minor, Penicillium notatum, Aspergillus ochrachrous, Candida pseudotropicalis and mycangial fungi #122 and #133.

Malt extract agar with 0.4, 0.8, 1.2, 1.6 and 2.0 mcg/ml of sorbic acid added was used to assay anti-fungal activity. C. minor, #122, and #133 were effectively inhibited but the yeast, Aspergillus, and Penicillium were not at 2.0 mcg/ml.

Using the same malt extract agar, Lotrimn® (clotrimazole) was used in 2, 4, 10, 20, 30, and 40 mcg/ml concentrations to test its inhibitory action on the above three organisms (yeast, Asp., and Pen.) Lotrimn® at a concentration of 30 mcg/ml effectively inhibited with growth of these three organisms.

Callow adults were placed in wells in artificial phloem media and capped over. This media contained 100 mcg/ml garamycin, 2.0 mcg/ml sorbic acid, and 30 mcg/ml Lotrimn®. As the beetles burrowed their way out of the capped wells, they were crushed in sterile saline and samples were placed in trypic soytone broth and thioglycolate broth. No growth was observed in any tubes. Thus preliminary results indicate that microbe-free SPB can be produced. Mass production techniques will be attempted in order to supply Dr. John Brand with beetles for pheromone analysis.